

Aminoglycoside-induced nephrotoxicity studied by proton magnetic resonance spectroscopy of urine

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Abstract

Background. Acute kidney injury is a relatively common complication of aminoglycoside therapy that affects 10–20% of patients receiving antibiotics of this class. Although the vast majority of patients do recover, the presence of certain risk factors may alter the clinical presentation and result in permanent renal damage. Thus, aminoglycoside-induced nephrotoxicity is a major concern and early diagnosis is critical. The aim of the present study was to characterize the early stages of aminoglycoside-induced nephrotoxicity using proton nuclear magnetic resonance (¹H NMR) spectroscopy of urine.

Methods. We studied 19 previously healthy patients who were hospitalized in our clinic due to bacterial infections. Combined antibiotic treatment with amikacin (1 g once daily) and a beta-lactam antibiotic was instituted in all patients. Urine and blood samples were collected before and after 5 days of aminoglycoside treatment.

Results. ¹H NMR spectroscopic data showed increased amounts of alanine and lactic acid (+138 and +255% compared to baseline values, respectively) and decreased hippurate (–50%) after aminoglycoside treatment. In addition, fractional excretions of sodium, magnesium and calcium were significantly increased (+271, +295 and +60%, respectively). These findings indicate that aminoglycosides can affect nephron tubules through two unrelated mechanisms that produce a partial ‘Fanconi-like syndrome’ and a ‘Barter-like syndrome’. However, because none of the study participants developed renal failure, these alterations are probably reversible and should not be used as sensitive or specific indicators of impending renal insufficiency.

Conclusions. Our study findings confirm that aminoglycosides can induce both proximal and distal renal tubular dysfunction. However, it remains unclear whether the early functional changes detected by ¹H NMR spectroscopy in patients treated with aminoglycosides are of prognostic value.

Keywords: aminoglycosides; proton nuclear magnetic resonance spectroscopy; tubular dysfunction

Introduction

Acute kidney injury is a relatively common complication of aminoglycoside therapy that affects 10–20% of patients receiving this class of antibiotics [1]. Intracellular accumulation of the drug in the proximal tubules may lead to cell necrosis, although the specific cellular mechanisms of aminoglycoside-induced nephrotoxicity have not been completely defined [2]. Distal nephron segments may also be affected during aminoglycoside nephrotoxicity. Clinically, aminoglycoside-related renal toxicity presents primarily as nonoliguric acute renal failure [3]. The earliest urinary manifestations of aminoglycoside-induced nephrotoxicity are an increase in urine output and the appearance of enzymuria, which represents the elimination of brush border membrane fragments or lysosomal enzymes [4] in the urine. In addition to these disturbances, various electrolyte abnormalities, such as hypomagnesaemia, hypokalaemia, hypocalcaemia and hypophosphataemia, may also occur [5]. These changes are probably secondary to impairments in tubular transport processes that occur during aminoglycoside-induced nephrotoxicity. Finally, serum creatinine and blood urea nitrogen characteristically increase 5–7 days after initiation of aminoglycoside therapy [6].

Recovery from aminoglycoside-induced nephrotoxicity is usually slow, particularly in elderly individuals. Plasma creatinine concentrations usually return to baseline levels within 21 days after cessation of therapy. Although the vast majority of patients do recover, the presence of certain risk factors (such as advanced age, presence of shock, high baseline creatinine clearance, female sex, history of liver disease and other characteristics) may alter the clinical presentation to result in an early appearance of acute renal failure as well as a prolonged course [7]. In addition, patients with underlying chronic kidney disease may experience incomplete recovery of renal function [8]. Therefore, these major pathologies associated with aminoglycoside-induced nephrotoxicity make it critical that early diagnosis is performed in clinical practice.

Proton nuclear magnetic resonance (¹H NMR) spectroscopy of urine provides an overall profile of low-molecular weight (LMW) metabolites that are characteristically altered during changes in physiological status, toxic insults

or disease processes [9]. For example, renal damage significantly alters the LMW metabolite profile in urine [10]. Furthermore, the nature of the metabolites detected can provide information about the localization of nephron injury [9, 11]. Previous studies using ^1H NMR spectroscopy have shown that aminoglycoside-induced nephrotoxicity involves both proximal tubule and distal renal tubule damage (loop of Henle and collecting duct) [12]. However, these studies were performed in critically ill patients with established renal failure [12, 13] who received numerous medications. Therefore, these patients provided inaccurate data on the renal changes associated with early stages of aminoglycoside-induced toxicity.

The present study was performed in patients with no clinical or biochemical signs of renal dysfunction. They had been hospitalized in an internal medicine clinic and had received an aminoglycoside. The aim of the study was to characterize the early stages of aminoglycoside-induced nephrotoxicity using ^1H NMR spectroscopy of the urine.

Materials and methods

Nineteen previously healthy patients with no comorbid conditions who were hospitalized in our clinic were enrolled into the study. They included 11 female and 8 male subjects, aged between 48 and 70 years, with a median 59 years. Indications for aminoglycoside treatment at the time of patient admission included acute cholecystitis (12 patients) and urinary tract infection (7 patients). We obtained a detailed medical history that focused on comorbid conditions that affect renal function and on the administration of other potentially nephrotoxic drugs. Thus, patients with impaired renal function [estimated glomerular filtration rate (eGFR) $<60\text{ mL/min/1.73 m}^2$], diabetes mellitus (fasting serum glucose $>126\text{ mg/dL}$), leukaemia, nephrolithiasis, cirrhosis, hyperbilirubinaemia (total bilirubin $>1.5\text{ mg/dL}$) and elderly patients (>75 years) with sepsis were excluded from the study. Patients receiving other potentially nephrotoxic drugs such as cyclosporine, cisplatin, lithium, diuretics, nonsteroidal anti-inflammatory drugs and drugs that inhibit renin-angiotensin axis activity (angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists) were also excluded from the study. Combined antibiotic treatment with amikacin (1 g once daily) and a beta-lactam antibiotic (usually a second- or third-generation cephalosporin such as cefoxitin or ceftriaxone, respectively) was given in all patients. In addition, forced hydration with $\sim 2\text{ L}$ of normal saline per day (or equivalent amounts of half-strength solutions) was instituted in all study participants. All study participants gave informed consent for the investigation, which was approved by the Ethical Committee of the University Hospital of Ioannina.

Urine and blood samples were collected before antibiotic administration and after 5 days of aminoglycoside treatment. More specifically, a spot urine sample that was equal in all patients was collected on admission before drug administration and on the 5th day of aminoglycoside treatment. The time of the baseline specimen depended on the time that patients were admitted, whereas on the 5th day of therapy, only morning spot urine samples were collected. Serum was separated by centrifugation at 1500 g for 15 min, followed by measurement of serum glucose, urea, creatinine, sodium, potassium, magnesium, calcium and phosphate on an Olympus AU600 Clinical Chemistry analyser (Olympus Diagnostica, Hamburg, Germany) using standard procedures. The uricase-peroxidase coupled reaction was used for quantitative determination of uric acid in serum and urine. Urine samples were centrifuged at 1000 g for 10 min, and an aliquot was taken for clinical chemistry tests (glucose, creatinine, total proteins, sodium, potassium, magnesium, calcium, phosphate and uric acid).

Sodium azide (1 g/L, 100 μL) was added to the remaining urine samples to prevent bacterial contamination and the samples were stored at -80°C until ^1H NMR analysis.

Fractional excretions (FE) of uric acid, sodium, potassium, calcium, phosphate and magnesium were calculated from the standard formula:

$\%FE_X = (U_X \times \text{Scr} / S_X \times \text{Ucr}) \times 100\%$, where S_X and U_X represent the serum and urine concentration of electrolyte X, and Scr and Ucr represent the serum and urine concentrations of creatinine [14]. FEs of potassium,

calcium, magnesium and phosphate that were $>13, 3, 4$ and 20% , respectively, were considered indicative of inappropriate urinary electrolyte wasting [15, 16]. GFR was estimated from serum creatinine levels using the Modification of Diet in Renal Disease Study equation:

$e\text{GFR} = 186 \times (\text{Scr})^{-1.154} \times (\text{age})^{-0.203} \times 0.742$ (if female), expressed in mL/min/1.73 m^2 , where Scr is serum creatinine in mg/dL , and age is expressed in years [17]. The renal phosphate threshold normalized for glomerular filtration rate (TmPO_4/GFR ; normal range $2.5\text{--}4.2\text{ mg/dL}$) was calculated according to the nomogram of Walton and Bijvoet [18]. Patients were diagnosed as having renal phosphate leak if they had hypophosphataemia with serum phosphorus $<2.8\text{ mg/dL}$ (0.9 mmol/L), a FE of phosphate $>20\%$ and a renal phosphate threshold of $<2.5\text{ mg/dL}$.

^1H NMR spectroscopy

Four hundred microliters of urine was mixed with 200 μL phosphate buffer (0.2 M $\text{Na}_2\text{HPO}_4/0.2\text{ M NaH}_2\text{PO}_4$, pH 7.4) in order to minimize pH variations and then a solution of 0.075% sodium 3-trimethylsilyl-(2,2,3,3- $^2\text{H}_4$)-1-propionate (TSP) in D_2O was added.

^1H NMR spectra were measured at 300 K on a 500 MHz Bruker DRX NMR instrument operating at 500.13 MHz and running on XWINNMR V.2.6 software. For the suppression of the water signal, the standard 1D pulse sequence NOESYPRESAT was used [19]. For each spectrum, 128 scans were collected into 64 K computer data points with a spectral width of 6009.6 Hz. The FIDs were multiplied with an exponential line broadening function of 0.3 Hz prior to Fourier transformation. The acquired NMR spectra were manually corrected for phase and baseline distortions with TopSpin 1.2 (Bruker Biospin Ltd) and referenced to TSP ($\delta^1\text{H}$ 0.0). The metabolites were identified according to published literature based on their chemical shifts and signal multiplicity. Quantification of selected metabolites was based on peak height measurements of selected signals [singlets (s) or doublets (d)] as follows: alanine at 1.48 (d), TMAO at 3.26 (s), glycine at 3.57 (s), citrate at 2.54 (d), lactate at 1.34 (d) and hippurate at 7.82 (d). The peak heights were corrected for the number of protons that formed each signal, normalized with respect to the methyl peak of creatinine (3.06 ppm) and were expressed as millimoles per mole creatinine. The above-mentioned metabolites were selected because they can be easily measured and they provide basic information about proximal and distal tubular function.

Statistical analysis

The Kolmogorov–Smirnov test was used to evaluate whether each variable followed a Gaussian distribution. Values are expressed as means \pm SD. Paired Student's *t*-tests were applied for comparisons between study parameters before and after treatment. Relationships among study variables were investigated using Pearson product-moment correlation coefficients (*r*). The level of significance was defined as $P < 0.05$.

Results

Laboratory characteristics of the subjects before and 5 days after treatment with aminoglycoside are shown in Table 1. There were no changes in eGFR or serum electrolytes after 5 days of aminoglycoside treatment, except for a significant reduction in blood urea levels. In contrast, the FEs of sodium, magnesium and calcium were increased after 5 days of aminoglycoside treatment compared to the values before treatment. However, there was no renal tubular wasting of phosphate (characterized by elevated FE of phosphate and reduced renal phosphate threshold) in the patients treated with amikacin. There was a nonsignificant increase in proteinuria on Day 5 of the treatment. Urinalysis failed to reveal pathological glycosuria or cylindruria after 5 days of treatment with amikacin.

Figure 1 shows spectra regions from the urine of a patient collected before and 5 days after aminoglycoside treatment. Because similar spectra were obtained from the other participants, these data are representative of the entire group. Compared with baseline, spectra from the 5th day of

Table 1. Laboratory findings from 19 patients before and after 5-day aminoglycoside treatment^a

Parameter	Before treatment with aminoglycoside Mean ± SD	After 5 days of treatment with aminoglycoside Mean ± SD	P
Serum			
Glucose (mg/dL)	111.2 ± 2.6	102.9 ± 2.0	NS
Creatinine (mg/dL)	0.9 ± 0.2	0.9 ± 0.3	NS
eGFR (mL/min/1.73m ²)	88 ± 16	87 ± 14	NS
Urea (mg/dL)	44.7 ± 21	25.2 ± 7.8	0.001
Uric acid (mg/dL)	4.4 ± 1.1	4.0 ± 1.3	NS
Potassium (mmol/L)	4.2 ± 0.5	4.4 ± 0.5	NS
Sodium (mmol/L)	140 ± 5.9	139 ± 2.8	NS
Magnesium (meq/L)	1.5 ± 0.2	1.5 ± 0.2	NS
Calcium (mg/dL)	8.8 ± 0.6	8.7 ± 0.4	NS
Phosphate (mg/dL)	3.3 ± 0.7	3.1 ± 1.5	NS
Urine			
FE uric acid (%)	11.1 ± 5.0	17.4 ± 18.0	NS
FE potassium (%)	10.4 ± 5.5	14.1 ± 8.2	NS
FE sodium (%)	0.7 ± 0.7	2.6 ± 3.9	0.05
FE magnesium (%)	2.2 ± 1.3	8.7 ± 7.1	0.02
FE calcium (%)	0.5 ± 0.2	0.8 ± 2.1	0.01
FE phosphate (%)	16 ± 10	18.1 ± 6.7	NS
TmPO ₄ /GFR (mg/dL)	2.9 ± 0.5	3.0 ± 0.7	NS
U _{TPR} /U _{CRE}	0.3 ± 0.1	0.5 ± 0.6	NS

^aTo convert to SI units: glucose in mg/dL to mmol/L, multiply by 0.5551; creatinine in mg/dL to μmol/L, multiply by 88.40; urea in mg/dL to mmol/L, multiply by 0.17; uric acid in mg/dL to mmol/L, multiply by 0.05948; calcium in mg/dL to mmol/L, multiply by 0.25; phosphorus in mg/dL to mmol/L, multiply by 0.3229. To convert from mg/g creatinine to mg/mmol of creatinine, multiply

treatment showed more intense alanine and lactate peaks but lower hippurate peaks. Six major metabolites were quantified from the ¹H NMR data and their mean ± SD concentrations are shown in Table 2. ¹H NMR spectroscopic data showed increased levels of alanine and lactic acid and decreased hippurate after 5 days of aminoglycoside treatment. The levels of glycine, TMAO and citrate were not altered by amikacin treatment. When the study participants were subdivided according to cause of admission (cholecystitis or urinary tract infection), there were no differences between these groups in aminoglycoside-induced changes in serum or urine parameters (data not shown).

There were no correlations between changes in FEs of electrolytes and changes in urine lactate, alanine or hippurate levels (data not shown).

The total duration of aminoglycoside therapy was 7–10 days in all patients. None of the participants developed acute renal failure during the course of the study.

Discussion

Aminoglycoside-induced nephrotoxicity is a relatively common complication in hospitalized patients that leads to significant morbidity and additional cost of therapy. Electrolyte abnormalities, enzymuria or abnormal urinary sediment with casts are only sometimes observed. In addition, serum creatinine does not provide a sensitive marker because aminoglycosides causes an initial proximal tubular dysfunction, which is not promptly reflected by serum creatinine levels. Because of these difficulties in early detection of aminoglycoside-induced nephrotoxicity,

new methods for the recognition of specific tubular disturbances may provide important help for everyday clinical practice.

In the present study, we evaluated renal tubular function of patients hospitalized in an internal medicine clinic who were treated with aminoglycoside (amikacin 1 g once daily) for at least 5 days. Tubular function was evaluated using conventional clinical measurements of serum and urine as well as with ¹H NMR spectroscopy of urine. At 5 days after initiation of treatment, there were no changes in serum creatinine or eGFR, indicating no reductions in renal function. Biochemical blood testing that included serum electrolytes failed to reveal significant differences in these parameters. Although our patients were haemodynamically stable upon admission, they may have had some contraction of the extracellular fluid volume due to the underlying infection, and this may have been the cause of the elevated blood urea levels.

The raised FE of sodium on Day 5 of the treatment may have been secondary to the intravenous administration of sodium-rich solutions or to subtle acute tubular disturbance caused by the aminoglycosides. However, in the present study, treatment with amikacin also caused magnesuria and calciuria. Observations in animals indicate that aminoglycoside treatment is followed by renal magnesium wasting, even in the absence of renal failure and abnormalities in renal tubular morphology [20–22]. Furthermore, previous findings indicate that the reabsorption of sodium, calcium and magnesium are closely linked in the thick ascending limb of the loop of Henle. In this same portion of the nephron, calcium-sensing receptors (CaSR) are present on the basolateral cell membrane [23].

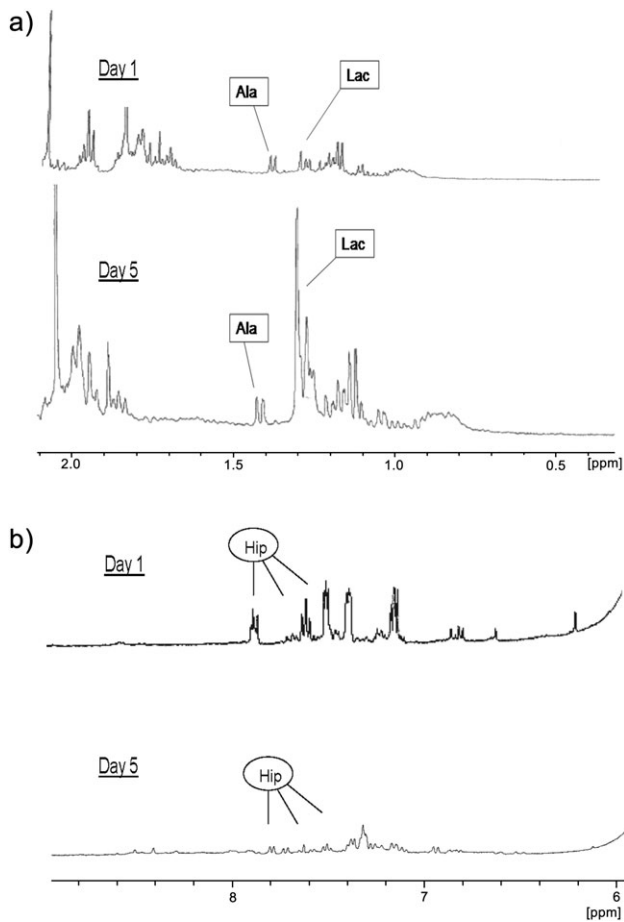


Fig. 1. Regions of ¹H NMR spectra (0.3–2.1 ppm and 6.0–9.0 ppm) obtained from the urine of a patient before aminoglycoside treatment (Day 1) and after 5 days of treatment (Day 5). An increased excretion of alanine (Ala) and lactic acid (Lac) (a) as well as a decreased excretion of hippurate (Hip) (b) after 5 days of treatment with an aminoglycoside was observed.

Table 2. Concentrations of the main urinary metabolites relative to creatinine (mmol/mol creatinine) of study participants before and after 5 days of aminoglycoside treatment

Metabolites (mmol/mol creatinine)	Before treatment with aminoglycoside Mean ± SD	After 5 days of treatment with aminoglycoside Mean ± SD	P
Hippurate	69 ± 36	34 ± 22	0.04
Glycine	253 ± 164	181 ± 126	NS
TMAO	19 ± 12	24 ± 21	NS
Citrate	147 ± 74	104 ± 55	NS
Alanine	14 ± 12	33.6 ± 23	0.04
Lactate	20 ± 27	71 ± 56	0.03

Aminoglycosides can stimulate CaSRs and lead to inhibition of different pathways that involve active sodium reabsorption. This inhibition reduces the luminal positive driving force and results in increased urinary calcium and magnesium excretion [22, 24]. Thus, the concomitant changes in renal calcium and magnesium homeostasis observed in our patients indicate that the distal nephron segments may represent an important target of aminoglycoside-induced

nephrotoxicity. This condition, termed in the literature as ‘Bartter-like syndrome’, has been reported in several animal and human studies [25, 26]. Proximal tubular cells also express CaSRs. The *in vitro*, administration of aminoglycosides initially causes alterations in cellular signaling and proliferation of cells that express CaSRs, and this is followed by apoptosis [27]. Furthermore, gentamicin-induced cell death can be prevented by administration of a CaSR antagonist [28]. However, the physiological role of CaSRs in the proximal tubule and its putative involvement in aminoglycoside toxicity remain unclear.

The great advantage of ¹H NMR spectroscopy is that it can quickly and noninvasively detect all the LMW metabolites found in urine. Most of these metabolites are not routinely determined in clinical practice despite their diagnostic potential for detecting tubular dysfunction. ¹H NMR spectroscopy of urine from our patients showed characteristic metabolic profile changes after 5 days of aminoglycoside treatment that were due mainly to increased levels of alanine and lactate and decreased hippurate. Neutral amino acids such as alanine are mainly reabsorbed by the sodium-dependent B⁰AT1 transporter, which is located in the S1–S3 segments of the proximal tubule [29]. Hippurate, which is synthesized in renal and hepatic mitochondria from glycine and benzoic acid, is secreted by the renal tubular cells and is continually excreted in the urine. A significant decrease in urinary hippurate may indicate insufficient proximal tubular secretion [30]. Finally, lactic aciduria has been linked to increased activity of anaerobic metabolic pathways, to decreased proximal tubular reabsorption and may also be a general marker of renal cortical necrosis [11, 31]. Thus, ¹H NMR spectroscopy of urine clearly demonstrates that aminoglycosides cause early effects on the proximal tubules. In fact, the pattern of selective aminoaciduria, lactic aciduria and depletion of urine hippurate detected in the present study indicate impairment of the transporting mechanisms located in the proximal tubular epithelial cells. However, the absence of renal glucosuria or renal phosphate or uric acid wasting indicates that aminoglycoside administration only partially affected proximal tubular function in our patients.

Taken together, our results indicate that aminoglycosides can affect nephron tubules through two independent mechanisms: an intracellular accumulation of the drug in the proximal tubules, which results in a partial ‘Fanconi-like syndrome’ as well as a ‘Bartter-like syndrome’ that can develop from aminoglycoside CaSR stimulation in the distal tubules.

Although we found that these ‘functional’ changes may represent the earliest stages of aminoglycoside-induced nephrotoxicity, their role in the prediction of significant renal failure remains doubtful. Our data indicate that forced hydration along with a timely discontinuation of the drug may reverse these alterations and preclude the evolution of the renal damage. In contrast with our findings, Le Moyec *et al.* [12] have shown that NMR spectra from cases of aminoglycoside-induced acute renal failure are characterized by elevated concentrations of metabolites that indicate damage to the medullar regions of the kidney [such as dimethylamine (DMA) or trimethylamine-*N*-oxide (TMAO)]. Interestingly, the ratio

of DMA to creatinine was the most important determinant of serum creatinine values in the population studied by Le Moyec *et al.* [12], whereas the TMAO levels in our patients did not change after amikacin administration. Thus, we suggest that in the absence of indications for structural damage in renal medulla, changes in renal tubule function detected by ^1H NMR spectroscopy are necessary but not sufficient indications of acute renal failure. Because these alterations are potentially reversible, they should not be used as sensitive and specific indicators of impending renal insufficiency.

In our study, aminoglycosides were coadministered with beta-lactam antibiotics (mainly a second- or third-generation cephalosporins) in all patients. These drugs are potentially nephrotoxic and may have contributed to the pathological changes observed in our study. However, it is well known that the most important mechanism for the renal toxicity associated with beta-lactams is a drug-induced interstitial nephritis. Although tubular dysfunction can be a complication of interstitial nephritis [32], none of our study participants developed characteristic findings of this syndrome (such as acute renal failure, rash and eosinophilia). Although it has been reported that cephalosporins can cause direct damage to renal tubules, the rarity of this phenomenon makes it unlikely that the drug contributed to the changes observed in our patients [33, 34].

In conclusion, we assessed tubular function in patients hospitalized in our department of internal medicine who were being treated with an aminoglycoside. In agreement with previous findings, we found that aminoglycosides can induce both proximal and distal renal tubular dysfunction. However, we found no correlation between changes in magnesium and calcium excretion (distal disturbance) with changes in urine lactate, alanine or hippurate (proximal disturbance). NMR-based urinalysis is a rapid and noninvasive technique that may contribute to the early detection of renal tubular dysfunction long before the onset of histopathological kidney damage, which is reflected by increased blood urea and creatinine levels. However, because none of our patients developed clinically significant renal failure, the prognostic value of the functional changes detected by ^1H NMR spectroscopy in patients treated with aminoglycosides remains questionable.

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Received for publication: 30.6.10; Accepted in revised form: 25.01.11
